Design and fabrication of a biomedical Lab-on-Chip system for SNP detection in DNA

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Abstract

A Lab-on-Chip system is proposed, capable of SNP (Single Nucleotide Polymorphism) detection in DNA. One of the core components is an advanced filter consisting of an ordered array of Si micro-pillars enabling fast and effective separation of 5 DNA segments with different length using chromatographic techniques. Also a dedicated micro-pump is fabricated based on conductive polymer actuation, generating the required high pressure to sustain the fluid flow through the total system. For the detector, a known detector principle is applied, but pronounced miniaturization is carried out in order to make a small and portable system.

Introduction

A SNP (Single Nucleotide Polymorphism) is a single nucleotide DNA sequence variation from the consensus sequence, which can result in differences in people's reaction to pathogens, chemicals, drugs, etc. Hence SNP genotyping gains importance in today's medical health care. Existing methods to detect SNPs use (larger) dedicated equipment, are often rather slow and need relatively large blood samples; also limited SNP specificity and sensitivity is an issue for some methods [1].

System description

In this paper, we propose a small Lab-on-Chip (LoC) system that enables fast SNP detection with high sensitivity and specificity, being very interesting for point-of-care applications such as personalized medicine. It functions as follows (Fig. 1): in the entrance unit blood is mixed with suitable reactants and pumped towards a thermal chamber for DNA extraction by a dedicated temperature treatment. By smart primer design, DNA segments of interest with respect to SNP detection are isolated [2]. Our detection system is designed to detect up to 5 SNPs. For each SNP under investigation, the corresponding DNA segment is given a different length: 100, 150, 200, 250 and 300 base pairs (bp) resp. These segments are further amplified by the on-chip PCR (polymerase chain reaction) system and pumped into the micro-pillar filter. When traveling through the filter, the DNA segments are separated due to their different length. By a system of valves and micro-channels each DNA segment will be transported to its SNP detector: a dedicated chamber in which the reaction of the DNA segment with a specific reagent will cause a change in acid concentration if the SNP is present. This concentration change can be detected by measuring the voltage over two electrodes present in the detector chamber.

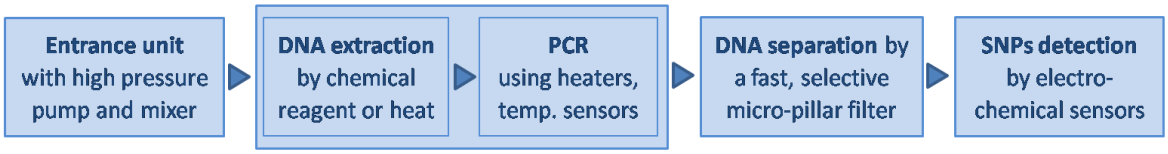

Figure 1: functional blocks of the SNP detection system

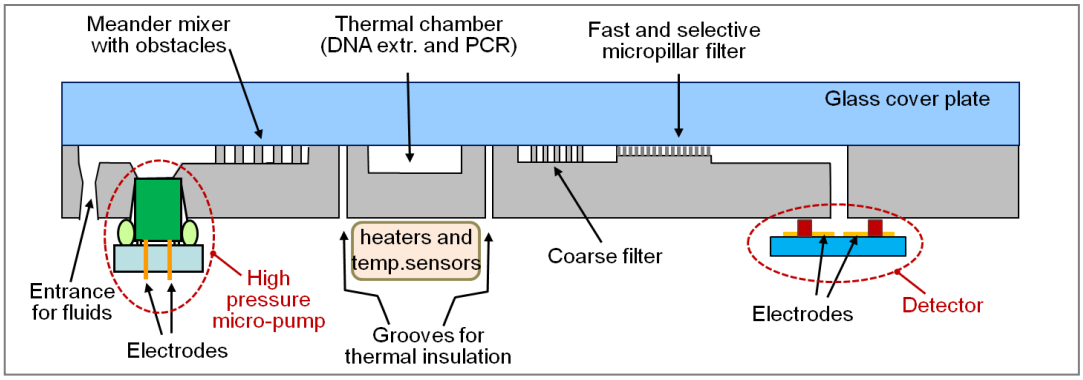

Figure 2: schematic cross-section of the SNP detection system.
Not visible: reservoirs containing chemical reagents and enzymes, and micro-channels and valves to combine the reservoirs with the main fluidic path. Also, the total device has 5 SNP detectors, only one is shown.

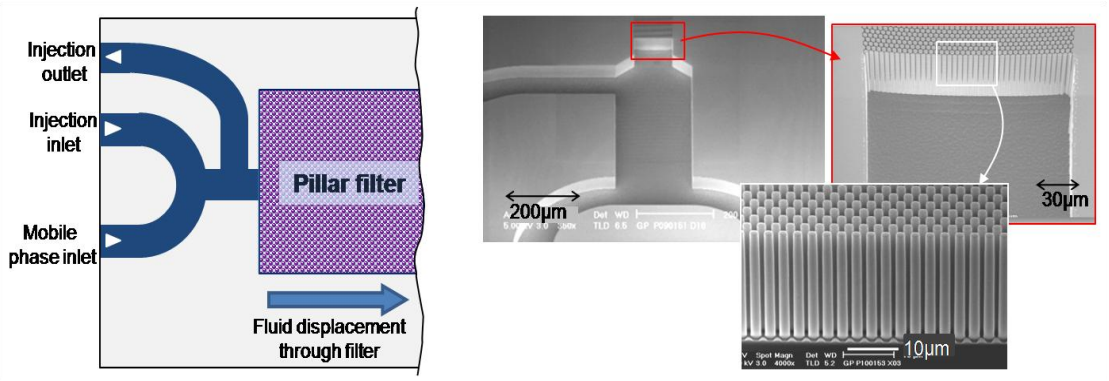

Figure 3: details of the micro-pillar filter, including wide channels and small pillars with high aspect ratio.
For this filter, pillars are 25µm high and 2µm wide, with an inter-pillar distance of 1µm. Both structures with very different dimensions and aspect ratio can be fabricated together due to optimized litho and etch processes.

Fabrication and component integration

The system fabrication is carried out mostly in a standard CR (clean room) using CMOS/MEMS processing techniques. The system consists of a main Si-based part and a few components are fabricated separately and integrated into the LoC system afterwards (See Fig. 2 for an overview of the total system).
One of the core components of the main Si-based part is a micro-pillar filter fabricated in a CR, simultaneously with other components such as micro-channels, mixers and a coarse filter. Another core component is the micro-pump which is fabricated in a lab environment and integrated in the total system. The last core component is the detector, made using CR processing but on a separate Si wafer; later the detectors will be filled with the suitable reagent for SNP detection and integrated into the total system. The total LoC system is currently under development: most critical components are fabricated and characterized (see further), other components are still under investigation.

The micro-pillar filter

The filter consists of an ordered array of pillars, which are several tens of µm high, and with a diameter and spacing in the µm and sub-µm range resp. The fabrication is not straightforward. DUV lithography is required to realize the small pillar dimensions [3]. Furthermore, since the filter is larger than the stepper exposure field, very accurate 'stitching' is needed. Finally, a new etch process was developed to enable the simultaneous etching of the micro-pillars with aspect ratio up to 30, and the wide (low aspect ratio) channels of the total fluidic system (Fig. 3). With our micro-pillar filter, we realized the separation of a mixture of 5 different DNA-segments in a very fast and highly selective manner (Fig. 4-5), even under isocratic conditions (constant concentration of mobile phase) and at room temperature, two conditions which will strongly facilitate the implementation of the pillar filter in the final portable SNP detection system.

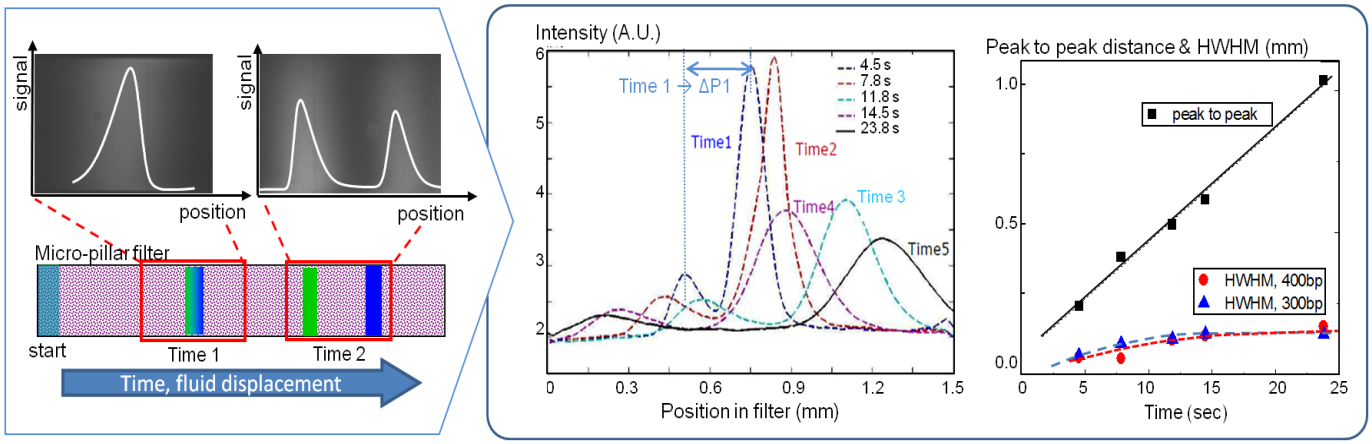

Figure 4: results of micro-pillar filter characterization: after injection of a mixture of 2 DNA segments (300 and 400 base pairs long), the fluid travels through the filter and separation peaks are obtained, which can be detected by a camera. These camera images are analyzed, peak to peak distance versus travel time and peak width (HWHM: half width at half maximum) is determined.
The filter quality is proven by the fact that the separation peaks appear very fast (less than 5 sec) and separate more with travel time, and that the peak width is broadening only slightly by further travelling through the filter.

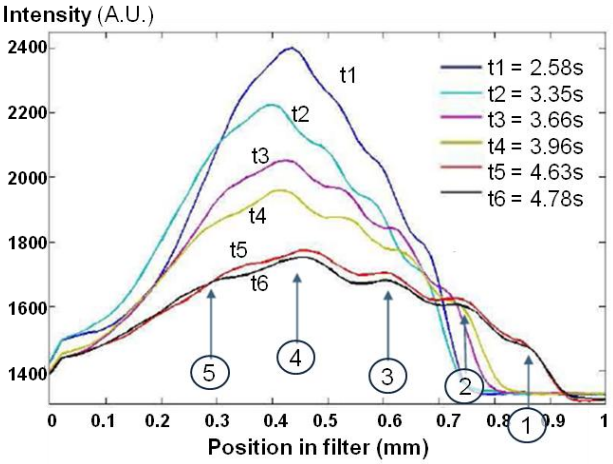

Figure 5: intensity plots showing the result of separation of a mixture of 5 DNA segments with length 100, 150, 200, 250 and 300 base pairs. The micro-pillars are 1µm wide and 10µm long and have an inter-pillar distance of 1µm. Good separation results are obtained working at room temperature and using isocratic conditions (Mobile phase: 10% acetonitrile and 90% water).

The micro-pump

A high pressure (MPa range) is required to pump the fluid mixture through a micro-pillar array, which is commonly accomplished using a large external pressure [4-7]. We realized on-chip high pressure generation by a micro-pump, advantageous for miniaturization and portability of our LoC system. A conductive polymer (CP) based actuator is fabricated using electrochemically deposited polypirrole (PPy) doped with Bis(trifluoromethylsulfonyl)imide) (TFSI) [8]. The actuator is made by stacking several base units, each comprising a PPy-TFSI layer, an electrolyte layer and a metal contact. It is placed in a protective polycarbonate case (Fig. 6).

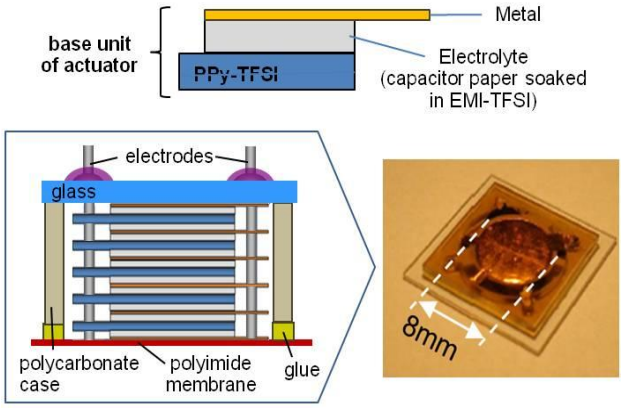

Figure 6: Top: the base unit of the actuator. Ions in the electrolyte are injected in or extracted from the conductive polymer, depending on the polarity of the applied bias. This causes a volume change. **Bottom:** schematic and photograph of the stacked actuator in its package. In order to generate fast and large displacement several base units are stacked together. The actuator is mounted in a polycarbonate case closed by glass (top) and a flexible polyimide membrane (bottom).

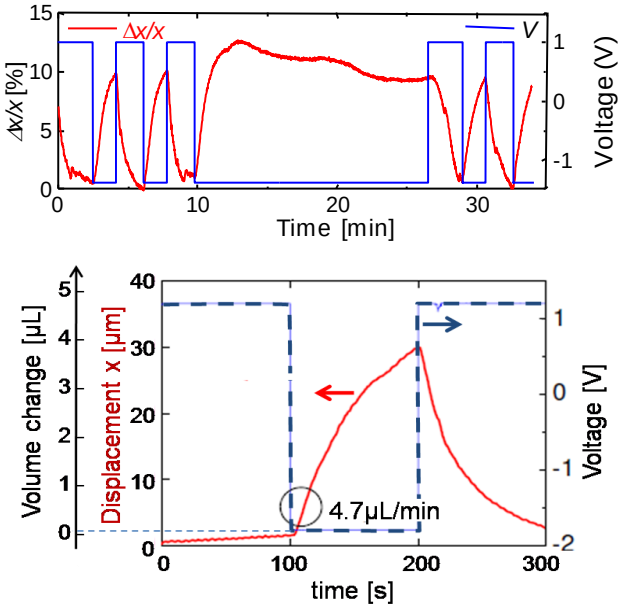

Figure 7: top: applied voltage and corresponding strain are plotted as a function of time for single layer actuator. **Bottom:** applied voltage and corresponding displacement and volume change for a stacked actuator (8 layers of PPy-TFSI, each 70 µm thick). The maximum obtained strain for the stacked actuator is 6%. Also indicated (see left axis) is the volume of fluid displaced by this actuator when connected to a micro-channel with a hydraulic diameter of ~200 µm.

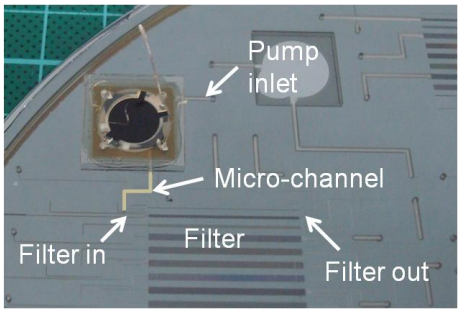

Figure 8: picture of the integrated system composed of the pump, the filter and channels.

Displacement tests without load showed that 13% strain is obtained by a single layer actuator and 6% by a stacked actuator (Fig. 7). Preliminary measurements under load indicate that very high pressures can be generated (range 10-20MPa). The actuator has been integrated with the microchannel chip (Fig. 8). An average flow of 2.6 µL/min was obtained when coupling the actuator to a capillary of ~180 µm hydraulic diameter (Fig. 9).

The SNP detector

The working principle of the detector is as follows: in presence of SNPs and through appropriate thermal cycles and chemical reactions, potassium ferrocyanide is generated when the SNP exists. This ferrocyanide is then detected electrochemically: in presence of an applied bias, ferrocyanides are reduced into ferricyanides and meanwhile release electrons, these electrons are detected as an electrical current by the external detector

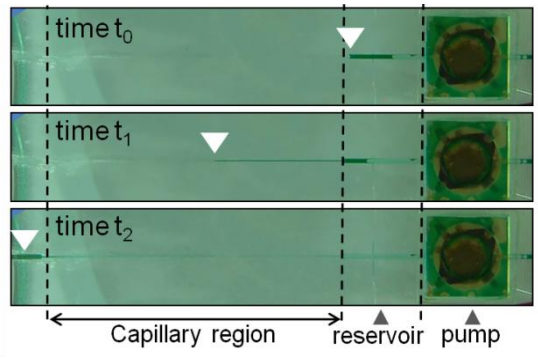

Figure 9: flow generation, the white arrow indicates the fluidic frontier. **At t_0** the liquid (dark green) is stored in a cylindrical reservoir near the pump. **At t_1** the bias voltage is switched on and the actuator expands. The liquid enters in the capillary region. **At t_2** the liquid has reached the cylindrical reservoir at the end of the capillary.

circuit. This detector principle has proven to be successful as described in previous work [2], although the tested SNP detector was realized on mm-scale. To fit in our LoC system, a detector sensitive to very small volumes (µl range) of DNA mixtures is essential, as it will determine the fluid volume on the chip, and hence the final device dimension.

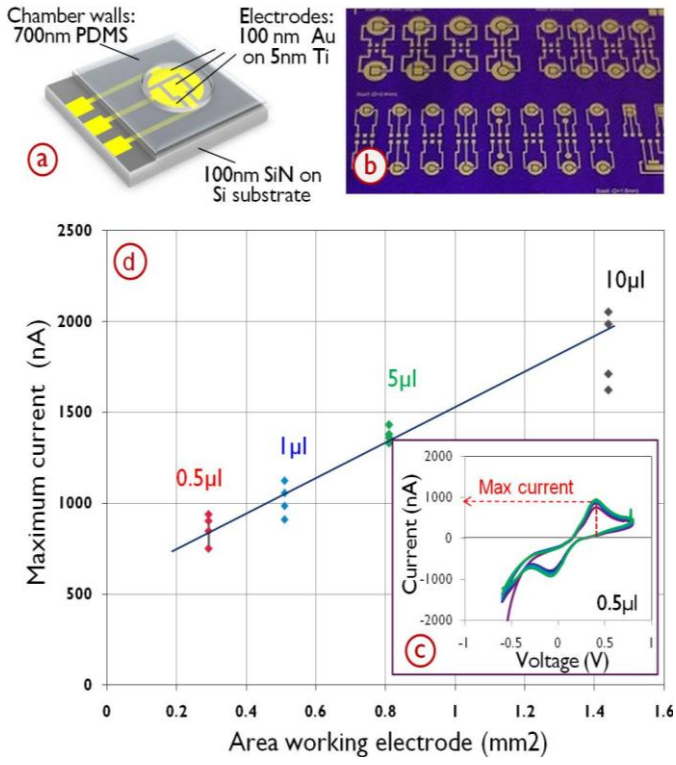

Figure 10: (a) schematic and (b) photograph of the detectors, having a micro-chamber size varying between 0.5 to 10 µl. (c) cyclic voltammetry measurements on the 0.5 µl sized detector (d) Peak current versus detector volume. The oxidation current depends linearly on the area of the working electrode.

The minimum detector size is determined by testing CR fabricated detectors with various sizes in the µl range. The detectors consist of a micro-chamber containing three SiN/Au electrodes (Fig. 10a,b). Using a 0.4mM ferrocyanide solution, a typical concentration under real operation conditions, cyclic voltammetry measurements are performed. Even the 0.5µl sized detector shows a well-defined redox current loop, (Fig. 10c), hence current measurements when applying one constant voltage will allow SNP detection, as shown in Fig. 10d. Using a volume of 0.5µl for SNP detection, the dimensions of the total LoC device can be as small as 2.5 x 4 cm^2.

Conclusions

A miniaturized SNP detection system is proposed. The core components are fabricated and characterized: (1) an advanced filter, consisting of an ordered array of Si micro-pillars enabling fast and effective DNA separation, (2) a dedicated micro-pump based on conductive polymer actuation, generating the very high pressure (range 10-20MPa) required to sustain a fluid flow through the total system, and (3) a strongly miniaturized SNP detector, based on electrochemical detection of potassium ferrocyanide. The combination of these core components enables the realization of a small and portable LoC SNP detection system, which will autonomously perform a fast and simultaneous detection of multiple SNPs.

References

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